Original Article

Attenuation of Residual Antibiotics Using Vitamin C in Homograft Heart Valves

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Highlights

- To use Vitamin C during processing of homograft heart valve can reduce the amount of residual antibiotics and prevents its consequences.

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ABSTRACT

Objective: The removal of residual antibiotics from whole homograft tissues after disinfection is a matter of concern. To avoid bacteriostasis, which causes falsely negative culture results, we investigated the effectiveness of a novel method applicable to whole homografts.

Methods: Thirty homografts, assessed as unsuitable for transplantation, were obtained from 24 deceased donors. Fourteen days after freezing, each homograft was thawed and divided lengthwise into 2 halves, with one serving as an unexposed control and the other as an exposed sample. Exposure was conducted using 400 µg/mL vitamin C. Five-step microbiological assays were performed using direct tissue samples and cryopreservation solution after thawing. The presence of residual antibiotics in tissue homogenates and cryopreservation solution was assessed by an agar diffusion test on seeded plates with 8 virulent strains. The effect of the intervention was determined by comparing the inhibition zones of exposed and unexposed tissue homogenates.

Results: Blood culture results from all donors were negative. The initial contamination rate was 13.3%, and the isolates were not detected after disinfection. Thawed tissues that were not exposed to vitamin C all yielded negative culture results, whereas one tissue sample exposed to vitamin C had a positive result (Pantoea eucrina). The diameter of the inhibition zone in seeded plates was 14.4% smaller with tissue exposed to vitamin C than that of controls (P=0.021).

Conclusion: The retention of antibiotics and carryover effect are unavoidable. Vitamin C can break down antimicrobial molecules and produce soluble by-products, which leads to a diminished amount of residual antimicrobials and a higher probability of detecting microorganisms in post-disinfection cultures.

Keywords: Homograft; Heart Valve; Residual Antibiotics; Decontamination; Vitamin C

RHYTHM
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Introduction

Annually, thousands of patients globally benefit from the replacement of structurally and/or functionally defective heart valves with homograft tissues. Therefore, donor screening and testing, as well as tissue processing, are critically important. In processing homograft heart valves, all tissue establishments adopt the most effective and least toxic decontamination method. These efforts aim to achieve a higher decontamination success rate and preserve tissue viability, which in turn leads to less structural deterioration and, consequently, extended durability .[1,2]

Because of the negative impact of terminal sterilization on tissue viability, antibiotic decontamination has been an integral part of homograft heart valve processing. Based on this principle and on prevalent virulent microorganisms cultured from procured tissues over time, tissue banks use unique antibiotic cocktails for heart valve decontamination, varying in antibiotic agents, number, dosage, incubation time, and environmental conditions. Consequently, each setting must adopt its own approach to manage different risks that could cause substantial adverse effects.[3-6]

In this context, low-dose antibiotic decontamination of homograft heart valves under variable temperature conditions is a widely established and effective procedure.[7–11] Nevertheless, the presence of residual antibiotics in decontaminated tissue and the likelihood of an inhibitory (carryover) effect from this inevitable remnant on microbiological assays are matters of concern and controversy, although the extent is unknown.[12–15] The potential life-threatening consequences of falsely negative culture results have led tissue bank specialists to consider first determining the presence of residual antibiotics and then adding a step to neutralize or remove them from decontaminated tissue valves before sampling for post-decontamination microbiological analysis.[16-18]

To attenuate antibiotic residues from decontaminated tissue grafts and prevent induced bacteriostasis or fungistasis, different approaches have been tried. Nonetheless, the effectiveness of these methods has not been extensively investigated.[19,20]

An additional concern regarding this topic is the potential for hypersensitivity reactions of varying severity following implantation of tissues containing residual vancomycin and amikacin, antibiotics used in combination with others as decontaminants in some tissue bank establishments. Still, because the most frequent and rare life-threatening reactions to vancomycin or amikacin are rate-dependent infusion reactions or dose-dependent reactions, and because severe vancomycin-induced hypersensitivity has not been reported after tissue allograft implantation, these adverse events are not of particular concern in this context. The primary concern remains bacteriostasis, which can result in the rare occurrence of early endocarditis.[7,16,21,22]

Considering the morphology and functional groups of the antibiotics used in combination as the decontamination cocktail in our setting—including hydrogen bond (H-bond) donor and acceptor potential and active acidic and basic sites—we hypothesized that antibiotic elimination could be effectively controlled by adjusting the pH value via a biochemical treatment based on a biological-grade weak organic acid designed to prevent drastic pH changes.[23-25]

In this context, cleavage of ether bonds can produce inactive compounds and soluble by-products that negatively affect cellular viability and the tissue scaffold.[26] Ascorbic acid (vitamin C), as a mild acid (pKa 4.17), is a suitable molecule for protonation of basic sites in decontaminating antibiotics. Moreover, ascorbate (the conjugated base of ascorbic acid) is suitably stable with a high tendency to lose H^+.[27] Vitamin C is also a well-known antioxidant that protects cells by scavenging free radicals; it therefore has a protective effect against nephrotoxicity resulting from oxidative stress due to administration of antibiotics, including aminoglycosides and vancomycin, in rat and human models.[28–31] Based on these properties, ascorbic acid—a water-soluble compound available as a sterile injectable medication—was chosen for this trial.

To validate the effectiveness of the antibiotic cocktail used for homograft decontamination, determine the presence of antibiotic residues that can interfere with sterility testing, and evaluate an additional step to attenuate antibiotic residues, the

present study assessed the effectiveness of ascorbic acid in attenuating residual antibiotics from homograft heart valves after antibiotic treatment.

Methods

Homograft Heart Valves

To undertake all aspects of tissue procurement and processing in the Iranian Tissue Bank (ITB), including donor assessment, screening, testing, and consenting,[32] 30 homograft heart valves assessed as not suitable for transplantation because of morphologic reasons or incompetence were used. These valves were used to determine the presence of residual antibiotics in processed homograft heart valves and to evaluate a new method to remove this remnant before post-disinfection microbiological analyses.

Tissue Procurement

Prior to tissue recovery, a blood sample from the donor was taken from a central vein for serological and microbial tests. Whole hearts were retrieved from deceased donors with circulatory cessation or from heart-beating brain-dead solid organ donors whose hearts were assessed as unsuitable for transplantation or for whom no matched recipient was found, provided echocardiographic studies demonstrated no valvular pathology. At retrieval, direct non-destructive sampling (6 tissue segments, 1 cm^3 each; the first sample set) was performed. The blood-free heart was placed in a sterile double plastic bag with 500 mL of cooled, sterile, antibiotic-free tissue culture medium. The bagged heart was then placed in a third bag, which was tied and labeled. The cool box containing wet ice (with the heart, blood samples, and culture tubes) was sealed and transported to the processing facility.

Tissue Processing

Immediate processing took place in class 100 clean conditions inside a laminar flow hood.[33] The heart was gently rinsed with cold antibiotic-free isotonic saline. The pulmonary and aortic valves were carefully dissected from the donor heart, measured, and sized. Valves were assessed for morphology and competency; valves that did not meet release criteria were used for the study. The second set of culturing was performed using six direct tissue samples. After rinsing, the valves were incubated and agitated in an antibiotic cocktail [250 mL of Medium 199 (M199; Pan-Biotec, Germany), 100 µg/mL of amikacin (Caspian Tamin, Iran), 100 µg/mL of vancomycin (Danapharm, Iran), and 200 µg/mL of fluconazole (Diflucan; Pfizer PGM, France)] at 22°C (room temperature) for 24 hours to reduce bioburden. Homograft tissues were then rinsed with sterile isotonic saline and stirred twice in tissue culture medium (M199) for 30 minutes each at 40 rpm; postdisinfection samples were then obtained (third set of culturing). The heart valves were placed in cryopreservation solution [85 mL of M199, 5 mL of albumin 5% (CSL Behring, USA), and 10 mL of dimethyl sulfoxide (DMSO) 50% (Merck KGaA, Germany)] at 4°C for 45 minutes. The heart valves and cryoprotectant solution were packed in a double sterile bag, and the bag was heat-sealed. The package was wrapped in aluminum foil. A controlled-rate freezer was used; the freezing process began with an initial rate of −1°C/min to −60°C, followed by a rate of −5°C/min to −120°C. Tissues were stored in the vapor phase of liquid nitrogen at −180°C for at least 14 days. After storage, the valves were thawed in and rinsed with 4°C sterile isotonic saline (twice with 300 mL) in class 100 clean conditions to remove the cryoprotectant. The heart valves were halved lengthwise, and the fourth set of samples was collected for microbiological testing and antibiotic detection from the control (unexposed) half. The fifth set of tissue samples was collected from the exposed half with the antibiotic removal solution.

Microbiological Assays

In each culturing set (first through fifth), six tissue samples (1 cm^3) were cultured in aerobic (Trypticase soy broth; TSB) and anaerobic (thioglycolate broth; Thio) bacterial and fungal (Sabouraud dextrose broth; SDB) culture media (Conda Pronadisa, Spain) and incubated at 22°C and 37°C for 14 days. An additional liquid sample (1 mL/culture medium) of cryopreservation solution was collected after thawing and cultured in the same manner as the tissue samples. Visible turbidity of the growth medium was considered a positive culture result. Microorganisms from positive samples were isolated by subculturing on

appropriate growth media.

Determination of Residual Antibiotics

To detect residual antibiotics in tissue samples, the agar diffusion test (agar well diffusion method) was performed.[19,34] Based on the microorganisms most prevalently isolated from contaminated tissues in our setting,[5] eight strains were selected: Staphylococcus aureus (ATCC6538), Staphylococcus epidermidis (ATCC12228), Streptococcus pyogenes (ATCC8668), Enterococcus faecalis (ATCC29212) ,Clostridium perfringens (ATCC13124), Klebsiella pneumoniae (ATCC13883), Pseudomonas aeruginosa (ATCC9027), and Candida albicans (ATCC10231). Depending on the selected microorganisms, different types of nutritious media were used where appropriate, including tryptic soy agar (Merck KGaA, Germany; with or without 5% defibrinated sheep blood for strains ATCC 6538, 8668, 29212, and 13124), nutrient agar (Merck KGaA; for strains ATCC 12228, 13883, and 9027), and Sabouraud 4% dextrose (Merck KGaA; for strain ATCC 10231). Bacterial inoculum was prepared by the direct colony suspension method. A few well-isolated colonies were suspended in 5 mL of distilled water to achieve a turbidity of 0.5 McFarland standard (equivalent to 1.5×10^8 CFU/mL).[35] Subsequently, on the same day of exposure, 2 mL of the suspension was mixed with 38 mL of autoclaved, cooled molten agar using the pour plate technique. In each 120-mm plate, four wells (4 mm each) were created (one in each quadrant): one well for tissue homogenate, one for cryopreservation solution, one for a positive control filled with antibiotic decontamination cocktail, and one for a negative control using antibiotic-free tissue culture medium. Each well was filled with 50 µL of tissue homogenate or liquid control. To prepare tissue homogenate after collecting direct samples for microbiological assay from each half (exposed and unexposed), the halves were blended with sterile isotonic saline (2 mL/g of tissue) (Omniblend I; JTC Electronics Corp, China) and centrifuged at 2500 rpm for 5 minutes (Eppendorf 5810 R; USA). The pellet (sediment) was used as a tissue homogenate. All Petri dishes were incubated at 37°C for 14 days under aerobic or anaerobic (CO_2 incubator for *C perfringens*) conditions. In all cases, after 24 hours (time 0) and 14 days, the inhibition zone (halo) was measured and recorded after subtracting the 4-mm well diameter.

Chemical Analyses

Ascorbic acid, acetic acid, citric acid, and formic acid were chosen primarily as acidic agents for antimicrobial protonation. The chemical behavior of these acids and the consequent change in pH, in combination with antimicrobials, were investigated. The changes in pH were assessed after the addition of these acids to the solution containing antimicrobials used as a disinfection cocktail. Because of the strong tendency of formic and citric acids to lose H^+, the decrease in pH was substantial (>2). In contrast, ascorbic and acetic acids did not change the pH substantially. For acetic acid, the presence of acetic acid and acetate (conjugated base) can act as a buffer to maintain a relatively constant pH, an important factor in pH-dependent biochemical reactions. Nonetheless, concerns regarding acetic acid included disproportionation reactions and by-products, such as CH_4 and CO_2, which harm tissue integrity.

Because ascorbic acid was considered the safest agent, reactions between antimicrobials—amikacin ($C_{22}H_{43}N_5O_{13}$; molar mass, 585.603 g/mol), vancomycin ($C_{66}H_{75}Cl_2N_9O_{24}$; molar mass, 1449.3 g/mol), and fluconazole ($C_{13}H_{12}F_2N_6O$; molar mass, 306.271 g/mol)—and ascorbic acid were performed. The antimicrobials were used at dosages similar to those in the disinfection solution, and the dose of ascorbic acid was gradually increased from 100 µg/mL to 400 µg/mL (1:1; equimolar amount) (uptitration method) with no substantial change in pH. In each experiment, based on the ascorbic acid concentration (100, 200, 300, and 400 µg/mL dissolved in 250 mL of distilled water), the mixture was stirred at room temperature at 80 rpm for 1 hour. No specific by-products were detected using gas chromatography–mass spectrometry.

Antibiotic Removal

The half subjected to exposure was placed in a bottle filled with 250 mL of sterile isotonic saline and 400 µg/mL of injectable vitamin C (Darou Pakhsh Pharmaceutical; Iran) and orbitally shaken at 250 rpm for 30 minutes at clean room temperature. The exposed half was then rinsed,

placed in another bottle filled with 250 mL of sterile isotonic saline, and shaken at 250 rpm for 15 minutes. The control half was rinsed, placed in a bottle filled with 250 mL of sterile isotonic saline, and shaken at 250 rpm for 15 minutes.

Bacterial Identification

The isolated bacteria were characterized phenotypically (cell morphology and biochemical reactions) using light microscopy and different culture media. Polymerase chain reaction (PCR) and 16S rRNA sequencing were performed to determine the strain isolated after exposure. Total genomic DNA of the isolated strain was extracted with the TOP General Genomic DNA Purification Kit (Topaz Gene Research Company; Iran). A pair of bacterial universal primers, 27f (5′-TTGGAGAGTTTGATCCTGGCTC-3′) and 1492r (5′-AGGAGGTGATCCAACCGCA-3′),[36] was used for the amplification of the 16S rRNA gene (bacterial ribotyping). PCR products were purified by Topaz Gene Research Company (Iran) and sequenced by Microsynth AG (Switzerland) in both directions. Sequencing data from bidirectional reactions were edited, and complete sequences were searched in GenBank (www.ncbi.nlm.nih.org; March 10, 2018) using the BLAST program[37] to identify the closest strain.

Antimicrobial Susceptibility Pattern

Antibiotic susceptibility of the isolate was studied on Mueller-Hinton agar using the disk diffusion method.[35] The diameters of inhibition zones were measured and reported after 24 hours of incubation at 37°C. Escherichia coli strain ATCC 25922 was used as quality control. Testing was performed for imipenem (10 µg), ciprofloxacin (5 µg), ceftazidime (30 µg), tetracycline (30 µg), and cefotaxime (30 µg) (Mast Group Ltd; Merseyside, UK) and piperacillin-tazobactam (110 µg) (Conda Pronadisa; Spain).

Statistical Analysis

Normally distributed data are described as mean (SD). Data not distributed normally are described by the median and range. Proportions (binomial variables) in two paired groups were compared using the McNemar test. The distribution of continuous variables was assessed for normality using the Shapiro-Wilk test. Continuous variables between two paired groups were compared using the paired t test for Gaussian distributions and the nonparametric Wilcoxon signed-rank test for non-Gaussian distributions. A 2-tailed P value of less than 0.05 was considered statistically significant. A 95% CI is provided for measures where applicable. Statistical analysis was performed using IBM SPSS Statistics, Version 23 (SPSS Inc, Chicago, IL).

Compliance with Ethical Standards

Homografts were sourced from Iranian donors in compliance with legal and ethical standards. Informed written consent and authorization were obtained according to national legislation and international guidelines for tissue retrieval, aiming for therapeutic purposes (implantation) from donors' next of kin. Additional consent was obtained for research purposes if the homografts were not suitable for transplantation. In practice at ITB, only tissues with excellent condition, in terms of morphology and competency, are released for transplantation. For the present study, homografts that were not optimal for transplantation were used because of anatomic defects or positive serological test results. The study methodology was approved by the ITB institutional review board.

The study was approved by the Research Ethics Committee, School of Medicine, Tehran University of Medical Sciences (approval ID: IR.TUMS.MEDICINE.REC.1404.105), under the title "Determination and Method of Eliminating the Residual Antibiotics from Homograft Heart Valves."

Results

Donors and Homografts

Thirty homografts (16 aortic and 14 pulmonary) were recovered from 24 deceased donors (16 heart-beating brain-dead and 8 non–heart-beating deceased donors with circulatory cessation). The mean (SD) age of tissue donors (83.3% male) was 32.3 (11) years. The median warm ischemic time of homografts was 3 hours (range, 1.5-20 hours). The mean (SD) cold ischemic time was 9.6 (1.4)

hours. Blood cultures were negative for all donors.

Microbiological Assays

The initial contamination rate (positive cultures on the first or second set of samples) was 13.3%; the isolated strains (Candida albicans and Enterobacter cloacae) were no longer detected after antibiotic disinfection. These initially contaminated tissues (n=4) were all retrieved from donors with circulatory cessation. The rate of positive postdisinfection culture (third set of culturing) was 6.7%, reflecting the rate of cross-contamination during processing or undetected initial contamination. These two valves contaminated with C albicans, which were different from the valves initially contaminated with this microorganism (postdisinfection positive cultures), were then separately treated with uptitration of fluconazole until apparent elimination at a dose of 1 mg/mL. There were no positive bacterial cultures among unexposed tissue samples (fourth set of culturing) or cultures of cryoprotectants after thawing; however, the rate in the exposed group was 6.7% (Table 1).

Validation of Antimicrobial Effectiveness Based on the mean diameter of the zones relating to control wells and compared with standard diameters set by antibiogram disk manufacturers, all studied strains were resistant to a widely established low-dose antibiotic disinfection cocktail (Figure 1).

Residual Antibiotics and Antibiotic Removal

Inhibition zones were observed to varying degrees in all plates except those seeded with Candida albicans. Exposure reduced the mean inhibition zone by 14.4%. The mean diameter of the inhibition zone of all seeded plates showed a statistically significant difference between unexposed and exposed halves (mean difference, 0.97 [SD, 0.93]; 95% CI, 0.197 to 1.75; P=0.02) (Figure 1). In addition, a significant difference was observed between the mean diameter of the inhibition zone at time 0 and day 14 (in both unexposed and exposed tissue samples) (P=0.02). The difference between the inhibition zone of cryopreservation solution and tissue homogenates was not significant (P=0.14). The study design and main findings are illustrated in (Figure 2).

Bacterial Identification and Antibiogram

The postexposure isolated strain were Pantoea eucrina (99% sequence similarity). The 16S rRNA sequence of the isolated strain is shown in (Figure 3).

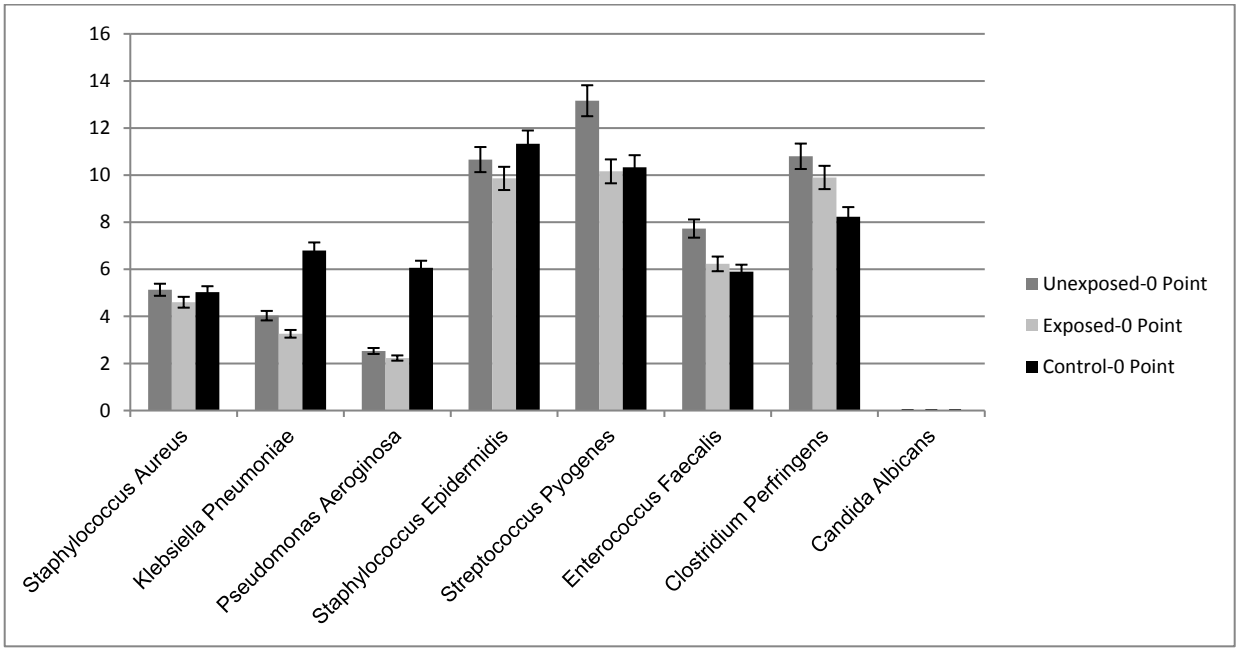

Figure 1. Mean diameter of the inhibition zone (mm) after 24 hours by strain. There is also a comparison of residual antibiotics in unexposed and exposed homograft tissue homogenates (n=30) (P=0.02).

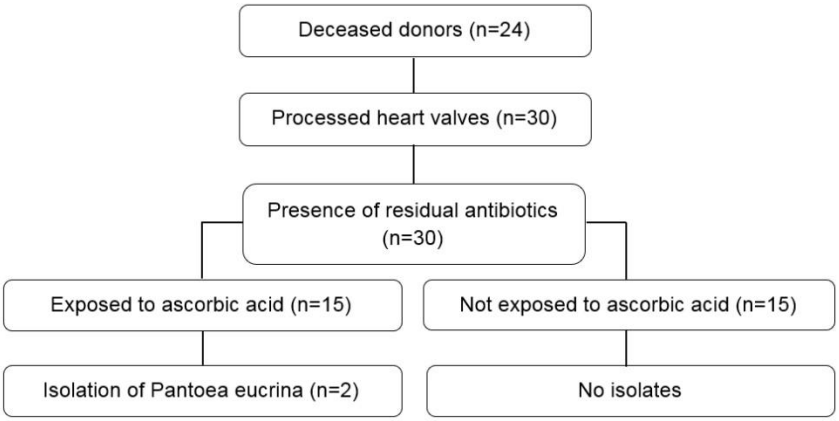

Figure 2. Experimental study on the effect of ascorbic acid on the removal of residual antibiotics in heart valve tissue.

Consensus sequence of P. Eucrina

GCTTGCTCCCCGGGTGACGAGTGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCCGGTGGAGGGGGATAACTACTGGAAACGGTAGCTAAT
ACCGCATAACGTCTTCGGACCAAAGTGGGGGATCTTCGGACCTCACGCCACCGGATGTGCCCAGATGGGATTAGCTGGTAGGTGAGGTAACGG
CTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTG
GGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAA
GGCGGCAAGGTTAATAACCTTGCCGATTGACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGC
AAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTTAAGTCAGATGTGAAATCCCCGGGCTTAACCTGGGAACTGCATTTG
AAACTGGCAGGCTTGAGTCTCGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGC
GGCCCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGA
CTTGGAGGCTGTTCCCCTGAGGAGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGA
ATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGCCTTGACATCCAGAGAACTTAGCA
GAGATGCTTCGGTGCCTTCGGGAACTCTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGA
GCGCAACCCTTATCCTTTGTTGCCAGCGGGTGATGCCGGGAACTCAAAGGAGACTGCCGGTGATAAACCGGAGGAAGGTGGGGATGACGTCAA
GTCATCATGGCCCTTACGGCCAGGGCTACACACGTGCTACAATGGCGCATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAG
TGCGTCGTAGTCCGGATCGGAGTCTGCAACTCGACTCCGTGAAGTCGGAATCGCTAGTAATCGTAGATCAGAATGCTACGGTGAATACGTTCCC
GGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCAAAAGAAGTA

Figure 3. Sequence map of Pantoea eucrina 16S rRNA generated with 27f and 1492r primers.

Table 1. Results of the microbiological analysis of 30 homograft tissues based on the culturing set

Set of Culturing	Initial Contamination (1st and 2nd) Recovery and predisinfection samples	Postdisinfection (3rd)	After Thawing		
			Cryopreservation solution	Unexposed (4th)	Exposed (5th)
Positive Culture (%)	13.3%	6.7%	0	0	6.7%
Isolate (risk)	Enterobacter cloacae (2/30), Candida albicans (2/30)	Candida albicans (2/30)	-	-	Pantoea eucrina (2/30)
Donor Type	Circulatory cessation	Brain death	-	-	Brain death

Discussion

Transmission of microbial infection by human tissue allograft is a life-threatening consequence.[17] The processing protocols used by tissue banks worldwide are designed to guarantee the safety and efficacy of homograft tissues. All routine measures are taken to eliminate microbial contamination and preserve cellular viability. Because of the presence of residual antibiotics[12] and the consequent risk of hidden, undetected virulent microorganisms in routine microbiological assays, the European and US pharmacopoeias mandate the elimination of all substances that may interfere with bacterial or fungal growth in sterility testing.[15] In contrast, Jashari et al[13] found no risk

associated with residual antibiotics in recipients. They also concluded that increased resistance in homograft recipients was attributable mainly to antibiotic retention in tissue.

Concordant with findings of previously published studies, the isolated pre-disinfection microbes in the present study were Candida albicans and Enterobacter cloacae.[5] This study indicates that the disinfection cocktail—considering parameters including types of antimicrobials, dosage, and incubation conditions—cannot reliably disinfect heart valves contaminated by staphylococcus aureus, staphylococcus epidermidis, streptococcus pyogenes, enterococcus faecalis, clostridium perfringens, gram-negative bacilli (in particular resistant strains of Klebsiella pneumoniae), and Candida albicans. Heart valves initially contaminated by these microorganisms should be discarded because disinfection can mask the contamination and even render pathogens resistant to postimplantation antimicrobial therapies.[17]

The findings of the present study regarding *Candida albicans* also suggest that a higher dose of antifungal (fluconazole) is required to apparently eliminate this yeast; however, the risk of cell toxicity and the likelihood of fungistasis make it advisable to discard tissues contaminated by this virulent microorganism at every step of procurement and processing. Current evidence suggests that residual antibiotics may cause false-negative culture results.

Exposure of tissues to ascorbic acid, a mild biocompatible acid, can reduce the amount of retained antibiotics. Consistent with the findings of Gatto et al[2] and contrary to the report by Buzzi et al,[15] our findings show partial antibiotic degradation after 14 days; however, residual antibiotics can still result in false-negative sterility tests.

With respect to antibiotic removal, Buzzi et al[15] used a RESEP tube to reduce retained antibiotics. Using these resin tubes reduced the inhibition zone by 81% to 98% and 94% to 100% on the initial and final day of the 14-day period, whereas in our experiment, the reduction following intervention was 14.4%. The post-thawing positive culture rate in their study increased by 50% using RESEP, whereas exposure to ascorbic acid in this study yielded only a 6.7% increase. Direct comparison is not valid; resin tubes are used only for liquids and homogenates and do not apply to whole homografts, unlike our approach.

The isolate in the present study, Pantoea eucrina, is a gram-negative, yellow-pigmented rod and a member of the Erwinaceae family from the order Enterobacterales. It has been associated mainly with catheter-induced bloodstream infection in rare cases.[38,39] This environmental pathogen was first isolated from human trachea in a nosocomial septicemia outbreak.[40] To date, there is no evidence of infection with this species following homograft heart valve implantation. Pantoea eucrina is sensitive to some antibiotics, such as gentamicin in combination with piperacillin-tazobactam.[40] The susceptibility of this strain was intermediate to imipenem, ceftazidime, and cefotaxime.

Limitations

There are limited approaches and studies within this topic, and further research with a larger sample size and adjusted data on a donor-source basis (brain-dead vs donors with circulatory death) could strengthen the evidence. Quantifying residual antibiotics using high-performance liquid chromatography could provide more objective data. The first through third sets of sampling, including indirect sampling (filter culturing method), could increase culture sensitivity. Studies using local microbial strains isolated from routine practice, rather than reference microorganisms provided by biobanks, could yield more validated findings. Identifying by-products resulting from reactions between ascorbic acid and antimicrobial agents by gas chromatography–mass spectrometry could determine the potential emergence of toxic substances. Postexposure cellular viability studies comparing unexposed and exposed tissue samples could be more indicative of the safety of the antibiotic removal procedure. Employing a method to validate the log reduction of infectivity of the strains used would help ensure high bacterial safety of the tissues.

Conclusion

Immersion and rinsing with a sufficient volume

of saline after tissue disinfection cannot eliminate excess antibiotics from homograft heart valves. Residual antibiotics resulting from the low-dose disinfection process can interfere with sterility testing, increasing the risk of transmitting bacterial or fungal infections to recipients because of false-negative culture results. To address this unavoidable risk, chemical interventions can potentially degrade the active molecules of these remnants. Evidence from the present study suggests that ascorbic acid can react with antimicrobials to produce soluble by-products, which can be removed by rinsing, thereby diminishing the risk of false-negative culture results.

Declarations:

Ethical Approval

The study was approved by the Research Ethics Committee, School of Medicine, Tehran University of Medical Sciences (approval ID: IR.TUMS.MEDICINE.REC.1404.105), under the title “Determination and Method of Eliminating the Residual Antibiotics from Homograft Heart Valves.”

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Conflict of Interest

The authors declare no conflict of interest.

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Author Contributions

E. Sepanian: Microbiological assays, systematic search, literature review, data interpretation, revision of the manuscript, and final approval of the version to be published.

O. Olang: Supervision of donor screening and tissue recovery, literature review, data registration, obtaining funding, revision of the manuscript, and final approval of the version to be published. M. Heydari Rouchi: Literature review, suggestion of removal methods, revision of the manuscript, performing chemical experiments, and final approval of the version to be published.

S. A. Tavakoli: Tissue procurement, technical supervision, critical revision, and final approval of the version to be published.

S. K. Hosseini: Tissue processing, microbiological assays, data acquisition, revision of the manuscript, and final approval of the version to be published.

H. Goodarzi: Tissue recovery, tissue dissection and sampling, revision of the manuscript, and final approval of the version to be published.

A. Heidary Rouchi: Conception and idea configuration, study design, statistical analyses, data interpretation, literature review, drafting the manuscript, responsibility for data integrity and accuracy of the data analysis, and final approval of the version to be published.

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